REMARKS

Status of the claims

Claims 28, 30-35 and 59-61 are pending in the application. Claims 28, 30-35 and 59-61 are rejected. Claims 28, 30, 32, 34, and 61 have been amended. Claim 62 is newly presented herein. No new matter is added.

Claim Amendments

Claims 28, 30, 32, 34, 59, and 61 are amended to overcome rejection under 35 USC §112. The term "universal" has been deleted from the claims. Claim 28 has been further amended to clarify the design of the probe used in the method of claim 28.

Claim 62 is newly presented herein. Claim 62 is drawn to describe the proximal and distal linkers, of the reporters of the instant invention, and is supported by Example 13 of the instant specification.

Claim rejection under 35 USC §112

Claims 28, 30-35 and 59-61 are rejected under 35 USC §112, first paragraph, as failing to comply with the written description requirement. The Examiner states that claim 28 incorporates apparently new matter in form of new language "two oligonucleotides overlapped end to end to form a linear probe" and that this is indefinite and lacks support in the instant specification. Similarly, the Examiner states that the phrase" a first universal probe linker on one end that hybridizes to a universal reporter linker of a reporter but does not bind the single stranded target sequence" also lacks support in the specification. Further, the Examiner states, that the phrase "a first universal probe linker on one end that hybridizes to a universal reporter linker of a reporter but does not bind the single stranded target sequence", lacks support for the negative limitation of not binding target sequence. Applicant respectfully traverses this rejection.

The Applicant submits amended claim 28, amended to recite "<u>overlapping oligonucleotide subunits which are joined together</u>". The instant specification describes said probe unit in detail (page 27 and 28, starting at paragraph 2, beginning at line 10 and Fig.3A-3D). The oligomers were made with a DNA synthesizer using phosphoramidite

chemistry. The primary probe subunit contains first linker sequences on the 5' end, which are complementary to the reporter, target specific sequences in the mid-portion, and second linker sequences at the 3'end that are complementary to the secondary probe unit. To facilitate hybridization, small sets of spacer bases (i.e., TTT) are commonly included in the primary probe unit to slightly separate the target specific segment from the two-linker segments. The secondary probe unit is generic and lacks target specific sequences, while its 3' end is complementary to the second linker sequences of the primary probe subunit, and its 5' end contains the first linker sequence complementary to the reporter unit. The 3' linker sequences are designed with 5' TA sites to facilitate psoralen plus UV crosslinking. Such crosslinking employs a bi- or tri- functional reactive psoralen compound which intercalates between paired bases, and, upon exposure to long wave UV light, forms a covalent bridge between thymidine bases that are adjacent to but opposite one another on facing complementary strands. The Applicants also submit Example 2 in the instant specification, which comprises of specific sequences and procedures that were effectively reduced to practice. The design, manufacture and application of WRAP-probes are further described in detail in Examples 5, 12, and 17. Structural features of WRAP-probe are described in detailed drawings seen in Figures 3A-3B, 4C, 7B and 14A.

Further, the Applicant submits that the term "universal" used in the instant claim has been deleted. The term "linker" is defined in the instant specification (Page 8, line 110) as "a single stranded nucleotide segment, **<u>that is not complementary to the target sequence,</u>** and which provides a means to bind probe and reporter components by virtue of complementary sequences in the linker." This definition clearly describes the negative limitation of the linker sequence that the Examiner contends was not evident from the examination of the instant specification. These are <u>generic linkers,</u> each consisting of a single stranded nucleotide segment, that is not <u>complementary</u> to the target sequence, and by this virtue, said linkers are unable to bind the single stranded target sequence. These sequences are checked against GenBank sequences and do not show complementarities to known natural sequences. These linker segments provide means to bind the probe (via complementary base pairing in the probe linker region) on one end and reporter components on the other end together by virtue of sequences in the reporter linker region, that are complementary to the probe linker in the probe and can be used to prepare

a number of probes. The method of the instant invention thus comprises: 1) the hybridization of the two-component probe unit to the target and 2) the secondary hybridization of the reporters via the reporter linkers to the probe linkers of the probe; to thereby create a linear complex of reporter-probe-reporter that is centrally wrapped around the target sequence. The reporters linked via the linkers to the probe are used for detection to indicate the target sequence and can be fluorescent, bioluminescent or any other molecule that can be used for detection.

Hence, the choice of the linker depends inherently on the target sequence being amplified and one skilled in the art would know to design a linker with a sequence that is unable to bind to the target sequence to be amplified but at the same time can be used to link the reporter to the probe. It is well known in the art that two strands of nucleic acid bind to each other via complementary base pairing hence, the Examiner's contention regarding the lack of support in the instant specification for the negative limitation of not binding to the target sequence is moot.

The Examiner further states, that "two oligonucleotides overlapped end to end to form a linear probe" is vague and indefinite since it is unclear if this means that the two oligonucleotides are simply hybridized to each other, whether there is a partial or a complete overlap. Further, "end to end" is unclear as the interaction intended is not clear. The applicant submits amended claim 28 supra. As described in the instant specification (Page 27 to 28, starting at paragraph 3, line 23 and Fig. 3B), the two oligonucleotides are hybridized via complementary base pairing between their linker regions. Hence, it is not a complete overlap but a partial overlap only in the specified regions. Accordingly, in view of the claim amendments and the arguments presented supra, Applicants respectfully request that the rejections of claims 28, 30-35 and 59-61 rejected under 35 USC §112 be withdrawn.

<u>The 35 USC §102 rejection</u>

Claims 28 and 30-32, are rejected under 35 USC §102(b) as being anticipated by **Wang** et al (U.S. Patent 4,925,785). The Examiner states that **Wang** teaches a method for detecting a target nucleotide sequence similar to the instant invention. Further, the Examiner states that **Wang** teaches the use of double stranded

reporter that is linked to a universal linker. In addition, the Examiner states that **Wang** teaches probes up to 300 bases long as well as formation of reporter array with multiple probes. Applicant respectfully traverses this rejection.

Applicant submits that the methods of **Wang** et al are significantly different than those employed by the instant invention. Examiner states **Wang** et al teach the step "b)" of the instant claim 28. Examiner states that **Wang** et al teach hybridizing the single stranded target nucleotide sequence with a nucleic acid probe unit (citing Fig 4C and Example 2, column 11 and 12, in **Wang** et al) where two oligonucleotides are overlapped (stating hybridized and citing Fig. 4C and column 12 in **Wang** et al), wherein the first oligonucleotide comprising three segments sequentially (<u>citing Fig. 16, which does not exist in **Wang** et al.</u>). In truth, the instant inventions claim 28 teaches in step "b) hybridizing the single-stranded target nucleotide sequence with a nucleic acid probe unit, comprising: two overlapping oligonucleotide subunits which are joined together to form a linear probe unit prior to target hybridization, wherein the first oligonucleotide comprises three segments sequentially:......." Hence, the instant invention teaches WRAP-probe comprising two overlapping oligonucleotides that are joined together **prior** to target hybridization. It's clear that the two-component linear probe unit, of the instant invention, is pre-manufactured as one unit prior to application.

Furthermore, **Wang** et al only teach a one-part single stranded probe, generally comprising two sequence segments, a target specific sequence and a linker sequence for joining to a polymer based reporter unit. In Fig. 4C **Wang** et al further depicts a probe with a second terminal linker sequence, but that sequence is not in fact joined to a second oligonucleotide, as the Examiner states, but rather the single oligonucleotide probe is joined to one or more double stranded DNA segments via a matching 5' or 3' single stranded linker end (Example 2 and Fig. 4C). Unfortunately, the simplistic drawing of Fig. 4C in Wang et al is confusing since it depicts these DNA segments (B' to D' and A' to C') as two short staggered lines that resemble two parallel oligonucleotides, one shorter than the other. However, **Wang** et al clearly identify these line segments in Fig. 4C as DNA segments (Example 2, paragraph 2) and describes these as a form of <u>reporter segments</u> in that they are hybridized to the probe after the probe has been hybridized to the target and that they are intended to cause detectable local gelatin in

the same manner as the polystyrene polymer reporter units described as the primary reporter unit for this method. It is notable that **Wang** et al only make claims regarding the reporter systems, and they make no claim of a probe structure. In addition, **Wang** et al use the common public domain term to describe their probes as "sandwich probes."

The method of **Wang** et al is further distinguished from the instant invention in that **Wang** et al teaches a probe collection (P_1, P_2......P_n) specific for the different sites in the target nucleic acid (T), wherein the different members of the probe collection of **Wang** bear common universal sequences (linker regions) A, B, C, and D that are complementary to universal sequences A', B', C', and D' attached to a large random coil polymer. In terms of procedure, **Wang** et al teach hybridization of these different probe units containing common linker regions first to the target DNA (column 11, beginning at line 60, Example 2 and Fig. 4A, 4B, and 4C). Following hybridization of the probe collection to the target sites, the unreacted probes are washed and then the entire probe-target complex is reacted with the polymer reporters to generate cross-linked networks of said reporters, and if sufficient cross-linking occurs, viscosity or other detectable changes like gel formation will occur. The method of **Wang** et al thus has practical limitations since there is a requirement for a measurable high viscosity or gelation i.e., many probe –reporter and reporter-reporter cross-links have to form and each cross-linked species has to have a high molecular weight, hence this methodology is only suitable for the detection of a large number of target sequences, and generally multiple different probes are required, each binding a different site within the target region (Example 2).

Structurally, the **Wang** method teaches a collection of different target-specific probes in which each probe comprises one oligonucleotide or one single-stranded segment of DNA. Accordingly, the probes in the collection differ in their target-specific sequences, but they all share common linker sequences. Such single-stranded probes by definition comprise one 3' end and one 5' end. In contrast, the instant invention teaches nucleic acid probe unit specific to a single target sequence that consist of two oligonucleotides that are overlapped and joined via two matching end linkers. Thus, unlike **Wang** et al who teach multiple probes that comprise one single-stranded component with a 3' and a 5' end, the instant invention teaches a two-part probe. The

probes of the instant invention by definition comprise either two 3' ends or two 5' ends, and if the same linker sequence is employed at both ends, then said probe unit can bind two copies of the same reporter unit. In the examples reduced to practice and recited in the instant invention, the probe units are prepared with two identical 5' linkers and DNA-based reporters called GeneTAGs are prepared with matching 5' linker on one or both ends so that a batch of identical reporters can be employed to bind said probes, one copy of said reporter binding to each end of the probe after the probe is bound to the target. Alternatively, arrays of double linker reporters can be employed with the probes of the instant invention. In contrast, the probe unit of **Wang** et al does not correspond to the structure and function of the probe unit of the instant invention and cannot be used to bind two copies of the same reporter unit. The present invention is also characterized by another important structural and functional feature in that after hybridization of the probe to the target and following the hybridization of the dual reporters to the probe, the resulting complex of the dual terminal reporters and a central probe unit comprises a linear, centrally wrapped reporter/probe/reporter complex that provides a partial helical enclosure of the target sequence midway along the length of the reporter/probe/reporter complex. This structurally advantaged binding mechanism allows for tethering multiple long reporters on a single target site. In effect the instant invention has no practical limitations in terms of detecting a large or small number of target sequences since detection is based on detecting signal from the attached dual reporters or arrays of said reporters, which provide multiple fluorescent labels or other signals.

Thus, the method of **Wang** et al differs considerably from the instant invention in both structural components and in the sequential steps of making and using the probes, the structural and functional characteristics of the probe and the dual reporter complex, and the potential applications of that reporter-probe-reporter structure. The instant invention has the capacity to detect a single target site in a single cell and has been effectively reduced to practice (Example 12 of the instant invention), wherein a single probe is bound to a single target and multiple reporters are bound by linker ends of the probe to create multi-reporter, reporter-probe-reporter complex that provide a partial helical enclosure of the target sequence, whereas **Wang** et al. teach a series of different probes and thousands or millions of target to enable detection (Example 2 of **Wang** et al.).

Further, the Examiner states that with regards to Claims 30, 31, and 32 of the instant invention, **Wang** teaches the use of a double stranded reporter that is linked to a universal linker, probes that are up to 300 bases long, and formation of reporter array with multiple probes. Specifically, the Examiner quotes column 6, lines 45-65 and Fig. 4C) with regards to claim 30. Applicant submits that column 6, lines 45-65 in **Wang** teach the nature of universal sequences employed by **Wang** et al. Specifically, lines 60-64 in Wang teach that a probe can have more than one attached universal sequence, one to direct attachment of the probe to a solid support and a second to enable attachment to a polymer molecule. Further, **Wang** teaches attachment of the probe to the random-coil polymer (reporter) is by covalent binding using known organic methods, or specific enzymes or nucleic acid binding proteins or via standard recombinant DNA procedures such as restriction-ligation recombinant DNA procedures (Column 7, lines 46-60).

The instant specification teaches reporters that are labeled double stranded DNA with single stranded linker regions on one or both ends that have the same polarity as the terminal linkers of the probes. Thus the same reporter unit can be attached to both ends of the two-part probe unit via complementary base pairing between their respective terminal linkers to form a linear reporter/probe/reporter unit that is centrally bound to the target sequence (Fig. 3A, 3B, and 3C). **Wang** teaches reporters that are random-coil polymers of high molecular weight such that each polymer molecule occupies large hydrodynamic volumes in solution (Column 7, lines 6-14). These polymers are selected from a group but not limited to commercially available synthetic polymers like polystyrene sulfonic acid, polystyrene malic anhydride, polysaccharides, polyglutamic acid, polyamino acid etc. (Column 7, lines 15-25). Biological polymers are also taught by **Wang** and include large DNA and RNA molecules. In addition, the detection of hybridization in **Wang** is based on whether the polymer is labeled or not. In the homogeneous assays, specifically Fig. 4C of **Wang** et al, as quoted by the Examiner, the polymer is not labeled but is attached to the probe, hybridization is detected based on changes in viscosity or gelation due to network formation. In contrast, the double-stranded DNA- based reporters of the instant invention are detected via labeled or modified bases that are incorporated into the reporter unit.

The probe unit taught by **Wang** contains both the probe and the universal linker sequences and is no more than 300 bases long. Claims 30 and 31 of the instant invention teaches a labeled reporter comprising of a double stranded polynucleotide sequence linked on one or both ends to a linker and is at least 100 bases long. The reporter of the instant invention does not contain any target specific sequences and thus cannot be defined as the probe.

With regards to claim 32 of the instant invention **Wang** teaches formation of a reporter array with multiple probes whereas the instant invention teaches formation of a reporter array via complementary base pairing within the reporter linker regions between multiple reporters. The instant specification teaches a probe unit synthesized as two overlapping oligonucleotide subunits joined together. The primary probe unit contains a first linker sequence on the 5' end which is complementary to the reporter linker sequence, followed by a target specific sequence in the middle, and a second linker sequence in the 3' end which is complementary to a second probe subunit. The second probe unit is generic and lacks the target specific sequences, while its 3' end is complementary to the second linker sequences of the primary probe subunit, and its 5' end contains the first linker sequence complementary to the reporter unit. This probe is distinguished by the initial formation of a probe that wraps around the target sequence at mid-point and by secondary binding of the reporters to the linkers. Hence, the array built up is due to attachment of multiple reporters to one probe thus amplifying the signal from the initial probe and reporter hybridization to increase the sensitivity of detection.

Hence, **Wang et al** teach a probe that is a single stranded piece of DNA or RNA, and not two overlapped oligonucleotides. Further, the so called universal sequences or linkers taught by **Wang et al** are a low complexity, highly repeated sequence which is greatly different from the unique, high complexity linkers of the present invention that also include multiple 5'TA sites for psoralen/UV crosslinking. Furthermore, the linkers taught by **Wang et al** are joined to the probe molecules by end ligation, as contrasted with the linkers of the instant invention, which are synthesized as a terminal part of the probe components. In addition, the reporter taught by **Wang** et al is a random coil polymer generally of polystyrene that can bind to itself as well as a probe, in order to create a large hydrodynamic volume in solution, a process that causes local gelatin, the means of

detection, and DNA or RNA molecules are considered substitutes for such chemical polymers, and if so, such DNA –based reporters are depicted by a double line if double stranded, which is the case of the segments of Fig. 4C identified as B' to D' or as A' to C'. Thus those segments are double stranded reporter molecules, added to the single stranded probe after it has hybridized to the target and washed, and there can be no basis for describing the DNA segments B' to D' or A' to C' as a second oligonucleotide component overlapped with the first oligonucleotide.

A claim is anticipated only if each and every element as set forth in the claim is found in the cited prior art reference. The identical invention must be shown in as complete detail as contained in the claim. Accordingly, in view of the arguments presented supra, Applicants respectfully request that the rejection of the claims 28 and 30-32, rejected under 35 USC § 102(b) as being anticipated by **Wang** et al be withdrawn.

Claim rejection under 35 USC §103

Claims 33-35 and 59-60, are rejected under 35 USC § 103(b) as being unpatentable over **Wang** et al (U.S. Patent 4,925,785) in view of **Urdea** et al (U.S. Patent 5, 681,697).

Examiner states that **Urdea** et al teach the use of reporter arrays to amplify signal for target detection. The Examiner also states that **Urdea** et al teach a first terminal probe linker and a second terminal probe linker and a direct interaction between reporter and terminal probe linker. Further, the Examiner states that **Urdea** et al teach a multi-linking unit which is double stranded in the interaction with the LE probe which is interposed between the reporter and linker and the terminal linkers, where the multi-linking unit comprises single stranded regions which hybridize with multiple reporter probes placed end to end which hybridize to the unit which is hybridized to the terminal linker and where there is a terminator or terminal reporter probe. Furthermore, the Examiner states, that many of the **Urdea** probes comprise a TA sequence including SEQ ID NO: 35, and that the spacer segments taught by **Urdea** comprise carbon. The Applicant respectfully traverses this rejection.

Wang et al and the Applicants invention are structurally and functionally distinct as discussed supra. The Applicant submits that **Urdea** et al only teach using a

probe with one terminal probe linker (see response {pg.14-15} filed February 11, 2005, for the Office Action of October 28, 2004). The probe (LE) taught by **Urdea** contains a L-1 region complementary to the target sequence and a L-2 region complementary to the label reporter or amplifier. This is consistent with what is shown in Figures 8, 11-13, and 15. Similarly, Figure 16 shows one LE probe with one terminal linker X and another LE probe with one terminal linker Y. The second vertical line connected to the LE probe in Figure 16 is not labeled as terminal linker and is not hybridized to any label reporter or amplifier. Hence, Figure 16 should not be interpreted as teaching two terminal linkers at both ends of the probe. Since, **Urdea** et al teach a significantly different probe composition than that of the instant invention hence, the rest of the teachings of **Urdea** et al. cannot apply to the instant invention. In view, of this a combination of **Wang** et al and **Urdea** et al does not make the claims of the instant invention obvious. Accordingly, Applicant requests that the rejections of claims 33-35 and 59-60, 35 USC § 103(b) as being unpatentable over **Wang** et al (U.S. Patent 4,925,785) in view of **Urdea** et al (U.S. Patent 5, 681,697) be withdrawn.

This is intended to be a complete response to the Office Action mailed June 07, 2006. Applicants submit that claims 28, 30-35 and 59-61 are in condition for allowance and respectfully request that claims 28, 30-35 and 59-61 be passed to issuance. If any issues remain outstanding, the Examiner is respectfully requested to telephone the undersigned attorney of record for immediate resolution. Should any fees be due, please debit Deposit Account No. 07-1185 upon which the undersigned attorney is allowed to draw.

Respectfully submitted,

Date:___________________

ADLER & ASSOCIATES
8011 Candle Lane
Houston, Texas 77071
Tel: (713) 270-5391
Fax: (713) 270-5361
BADLER1@houston.rr.com

____
Benjamin Aaron Adler, J.D., Ph.D.
Registration No. 35,423
Counsel for Applicant